TMH/DAG:lam 11/03/03
PATENT

Attorney Reference Number 4239-61302
Application Number 10/017,372

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 3, line 1 with the following rewritten paragraph:

There have been a few reports of TGF-β fusions in the literature, but the described molecules have been essentially biologically non-functional. In an effort to produce large quantities of easily purified TGF-β that retained activity, Nimni and co-workers expressed 6x His-tagged TGF-β fusion proteins in *Escherichia coli* (Tuan *et al.*, *Conn. Tiss. Res.*, 34:1-9, 1996; Han *et al.*, *Prot. Expr. Purif.*, 11:169-178, 1997). Serious difficulties were encountered in refolding the denatured fusion protein, and full biological activity was not retained using this system. In addition, the Nimni constructs cannot be used to express a tagged TGF-β in a mammalian host, since the constructs lack a part the TGF-β pro-protein (the LAP), which is essential for secretion and proper folding of the TGF-β protein. In an earlier effort, Wakefield *et al.* (*Growth Factors*, 5:243-253, 1991) reported attaching an endoplasmic reticulum retention signal ~~(KDEL)~~ to the C-terminus of full-length TGF-β1, in an attempt to maintain the protein in the cell (rather than secreting it to the extracellular matrix). This construct had no biological activity.

Please replace the paragraph beginning at page 5, line 3 with the following rewritten paragraph:

FIG 6A illustrates the design of TGF-β1 with an N-terminal FLAG tag (NFLAG-TGF-β1). The FLAG tag (boxed sequence) (SEQ ID NO: 7) was inserted immediately following the cleavage site (indicated by arrow). Amino acid sequence shown above nucleotide sequence in single letter code (corresponding to nucleic acid residues 817-954 of SEQ ID NO: 8).

Please replace the paragraph beginning at page 5, line 17 with the following rewritten paragraph:

FIG 6C shows portions of the sequences of N+5FLAG-TGF-β1 (residues 815-914 of SEQ ID NO: 32) and N+5HA-TGF-β1 (residues 815-914 of SEQ ID NO: 36). Only the region in the vicinity of the cleavage site is shown; the numbering is different than in the attached

sequence listing and refers to the position of the illustrated residues in the plasmid. The epitope tag sequences are boxed. Arrows indicate cleavage site that separates LAP from the mature TGF-β peptide. Note the localized concentration of basic amino acids in the region immediately upstream of the cleavage site (RHRR) (residues 275-278 of both SEQ ID NOs: 33 and 37).

Please replace the paragraph beginning at page 15, line 20 with the following rewritten paragraph:

One representative translocation/transduction peptide (which serves to facilitate translocation of a protein into a cell) is the Tat_{49-57} ~~(RKKRRQRRR)~~ fragment from the human immunodeficiency virus (HIV). See, for instance, Vecero-Akbani *et al.* (*Methods Enzymol.* 322:508-521, 2000); Falnes *et al.* (*Biochem.* 40:4349-4358, 2001); and Becker-Hapak *et al.* (*Methods* 24:247-256, 2001). Antibodies are available and can be produced that recognize this peptide, and so it can be viewed as a multi-functional peptide.

Please replace the paragraph beginning at page 63, line 29 with the following rewritten paragraph:

Flow cytometry. Mv1Lu cells were harvested using CellStripper™ ~~(Manufacturer and location?)~~, a non-enzymatic dissociation solution. Cells were counted and 3 x 10^6 cells were transferred to 5 mL FACS tubes. Cells were washed with PBS and fixed with 4% buffered paraformaldehyde on ice, for 5 minutes. The paraformaldehyde (8%) was added dropwise to cells in an equal volume of PBS with gentle vortexing to avoid clumping of cells. Cell pellets were then washed with 4 mL of cold PBS. Cells were permeabilized by the addition of 1 mL of -20C methanol with vortexing and incubated on ice for 2 minutes. Cells were washed with PBS and then treated for 5 minutes at room temperature with 50 mM glycine in PBS (to quench auto-fluorescence). After washing with PBS, cells were pelleted and 200 µl of blocking buffer plus 20 µl of conditioned medium containing N+5FLAG- or N+5HA-TGF-β1 ligand were added. Cells were incubated with ligand for 2.5 hours at 4° C. Following incubation with ligand, cells were washed two times with ice-cold PBS and once with blocking buffer. Cells were then incubated with blocking buffer for 30 minutes at room temperature prior to addition of 200 µl of blocking buffer containing primary antibody (anti-FLAG, anti-HA monoclonal or anti-KLH isotype control) diluted 1:1000 (approximately 0.4 µg in 200 µL). Incubation with primary

antibody was carried out at 4° C overnight. The following day, samples were washed twice with ice-cold PBS and once with blocking buffer. Samples were then incubated for one hour with TRITC-conjugated goat anti-mouse (Jackson Immunolabs) at 1:100 in blocking buffer. After washing twice with PBS and once with FACS buffer (PBS + 1.0% heat-inactivated FBS), samples were resuspended in 400 µl of FACS buffer and analyzed by flow ~~cyometry~~ cytometry using a FACSCalibur (Beckton Dickinson).

Please replace the paragraph beginning at page 70, line 1 with the following rewritten paragraph:

Table 2

Fusion name and SEQ ID NOs.	5'UTR	CDS	3'UTR	AA 1-5 of mature TGF-β	Epitope tag	Mature fusion
N+5FLAG-TGF-β1 (NOs: 16 & 17)[1]	1-347[2]	348-1559 (1-404)[3]	1560-1612	1182-1196 (279-283)	1197-1220 (284-291)	1182-1559 (279-404)
N+5HA-TGF-β1 (NOs: 20 & 21)	1-347	347-1571 (1-408)	1572-1624	1182-1196 (279-283)	1197-1232 (284-295)	1182-1571 (279-408)
N+5FLAG TGF-β2 (NOs: 24 & 25)	N/A	1-1284 (1-428)	N/A	907-921 (303-307)	922-945 (308-315)	907-1284 (303-428)
N+5HA TGF-β2 (NOs: 26 & 27)	1-7	8-1303 (1-432)	N/A	914-928 (303-307)	929-964 (308-319)	914-1303 (303-432)
N+5FLAG TGF-β3 (NOs: 28 & 29)	N/A	1-1272 (1-424)	N/A	895-909 (299-303)	910-~~945~~933 (304-311)	895-1272 (299-424)
N+5HA TGF-β3 (NOs: 30 & 31)	N/A	1-1284 (1-428)	N/A	895-909 (299-303)	910-945 (304-315)	895-1284 (299-428)
N+5FLAG TGF-β1 (NOs: 32 & 33)	1-10	11-1222 (1-404)	1223-1349	845-859 (279-283)	860-883 (284-291)	845-1222 (279-404)
N+5FLAG-TGF-β1 (NOs: 34 & 35)	1-14	15-1226 (1-404)	1227-1253	849-863 (279-283)	864-887 (284-291)	849-1226 (279-404)
N+5~~FLAG~~ HA TGF-β1 (NOs: 36 & 37)	1-10	11-1234 (1-408)	1235-1361	845-859 (279-283)	860-895 (284-295)	845-1234 (279-408)
N+5~~FLAG~~ HA TGF-β1 (NOs: 38 & 39)	1-10	11-1234 (1-408)	1335-1361	845-859 (279-283)	860-895 (284-295)	845-1234 (279-408)

[1]Refers to the nucleic acid sequence and amino acid sequence for the listed fusion.
[2]Residue positions correspond to the position in the nucleic acid sequence.
[3]Residues within parentheses correspond to the positions in the amino acid sequence.